

**ADAPTIVE PLASTICITY IN RESPONSE TO POPULATION DENSITY AND NITROGEN  
FERTILIZATION IN (Thorn Apple (*Datura stramonium* L.)**

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**ABSTRACT**

Phenotypic plasticity is a flexible strategy that allows a genotype to produce different phenotype in heterogeneous environments. This could be an important determinant of the ability of a colonizing weed to inhabit novel environment. Assessing the amplitude of plasticity in a colonizing weed could help to development management strategies for its Control as well as, developing predictive models for its spread. In this study, phenotypic plasticity in response to variations in density and nutrient supply in *Datura stramonium* (L.) was measured in two field experiments. Seeds of the species were sown at inter-plant distance of 7cm, 14cm and 21cm to represent high, medium and low density, respectively. Plants in the other experiment received Nitrogen fertilization at the rate of 80, 100 and 120kg/h to represent low, medium and high nutrient supply, respectively. Plants sown at high density showed significant increase in traits such as specific leaf area, leaf area ratio and number of leaves; traits that are associated with light capture. Similarly, plants sown at low nutrient availability have significantly higher root biomass, root mass ratio, and root: leaf length; traits that will increase the potential for the absorption of nutrients below the ground. We interpreted these results as a strategy for this species to maximize fitness in environment of low light and low nutrient availability. Our study also highlights the importance of considering a wider range of plant traits to fully understand the ability of plants to respond to changes in the environment.

**Key words:** *Datura*, density, flexible strategy, nutrient, plasticity, specific leaf area.

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## INTRODUCTION

Phenotypic plasticity is the ability of an organism or a genotype to produce different phenotypes depending on the environment in which it grows (Nicrota *et al.*, 2010). Changes in traits expression in different environments could be as a result of adaptive or passive plasticity (Sultan and Bazzaz, 1993). Passive plasticity is change in trait expression as consequence of changes in overall plant size due to differences in resource availability among environment (Coleman *et al.*, 1994). Adaptive plasticity on the other hand, is a change that mitigates the effect of the changes in the environment (Wright and McConnaughay, 2002). Differentiating between the two kinds of plasticity would enhance our understanding on the evolution of plasticity (Nicrota *et al.*, 2010).

There is an emerging consensus amongst researchers that colonizing weeds are able to inhabit novel areas by exhibiting phenotypic plasticity in ecologically important traits (Mal and Doust-Lovett, 2005; Huang *et al.* 2009). Adaptive plastic responses in colonizing weeds may often help them maintain function and fitness across a range of environments, and influence their ecological breadth and response to variations in crucial environmental requirements (Sultan, 2000;

Daehler, 2003). This suggests that adaptive plasticity could be an important predictor of invasiveness (Meekins and McCarthy, 2000; Claridge and Franklin, 2002), and an understanding of a weed's plastic responses could be crucial in developing strategies for its management (Mal and Doust-Lovett, 2005).

The common thorn-apple (*Datura stramonium* L.) is an annual plant that is invasive in almost all temperate and tropical regions of the world (vanKluenen *et al.*, 2007). The species was listed as one of the invasive alien plants in China, and have been found to be invasive in twenty seven provinces including the northeast provinces (Weber *et al.*, 2008). In Zhongmachang, Changling County of Jilin Province, Northeast China, this species was relatively rare until the year 2000 when significant increase in population was noticed (Zhang Boatian 2007, personal communication). Because phenotypic plasticity is an important indicator of a species ability to colonize a new environment, *Datura stramonium* is expected to exhibit significant plasticity in the expression of one or more traits in response to variations in environmental conditions. In this study, we investigated the extent of plasticity in *Datura stramonium* by experimentally manipulating population density and nutrient supply in the field.

Although field experiments face problems in separating the specific effects of multiple growth variables on the target species, they have the advantage of staying close to realistic interactions between plants and their environments (Bell and Galloway, 2007). Competition due to increased density is one of the important environmental factors influencing growth and trait expression in natural populations (Bouvet *et al.*, 2005). High population density could result to reduction in resources such as light, water, nutrient availability, and this could elicit plastic responses in plant populations (Franklin and Whitelam, 2005; Bell and Galloway, 2007; Weiner *et al.*, 2009; Japhet *et al.*, 2009). Plasticity has most often been described in terms of the relative allocation of biomass between two organs in a plant, for example, allocation of biomass between vegetative structures and reproductive structures (Weiner *et al.*, 2009). Plasticity studies testing a broad range of structures have received less attention. Considering a wider range of plant traits could bring more precision in predicting the range of habitats a colonizing weed can inhabit.

In this study, we addressed the following questions:

(1) Does *Datura stramonium* modify its traits in response to variations in density and nutrient supply?

(2) Do these changes relate to adaptive plastic responses or passive plasticity?

## MATERIALS AND METHODS

### Study Species

*Datura stramonium* is a leafy annual which can reach up to 2 meters in height. The plants produce spiny seed pods and large white or purple trumpet-shaped flowers. Most parts of the plants contain atropine, Scopolamine, and hyoscyamine. *Datura* has a long history of use both in South America and Europe and is known for causing delirious states and poisonings in uninformed users. The leaves are alternate, 10-20 cm long and 5-18 cm broad, with a lobed or toothed margin. The flowers are erect or spreading (not pendulous like those of the closely allied Brugmansia), trumpet-shaped, 5-20 cm long and 4-12 cm broad at the mouth; colors vary from white to yellow, pink, and pale purple. The fruit is a spiny capsule 4-10 cm long and 2-6 cm broad, splitting open when ripe to release the numerous seeds.

### Experimental Design and Treatments

Two field experiments were conducted in 2007 at the Experimental Station of Northeast Normal University, located at Changling County Zhongmachang (44°30'~44°45' N, 123°31'~124°10' E) of Jilin

Province, China. Treatments were chosen to generate a wide range of growing conditions as in Sadras *et al.* (1997); Bonser and Aarssen, (2003); Wang *et al.* (2006). Experiment 1 compared plants sown at three different densities; Experiment 2 compared plants sown at three levels of nutrient availability. In Experiment 1, different densities were achieved by manipulating plant to plant distance; 7cm, 14cm and 21cm to represent High, Medium and Low density respectively. In Experiment 2, plots received N fertilization in the form of NPK at the rate of 80, 100 and 120kg/ha to represent Low, Medium and High nutrient supply respectively. In all the experiments, treatments were laid out in a completely randomized design with three replicates. Plot size was 2x3m. Both experiments were carried out in adjacent area of the same field. In Experiment 2, the distance between plants was 14cm. Undesired weeds were Controlled mechanically by hoeing.

### Measurements

To Control for ontogeny, we randomly harvested six plants per each replicates and per each treatment (N=18) at maturity. Plants were dug up to a depth of 50cm to get most of the root samples. For each plant, the following morphological and biomass traits were recorded: Plant height, Number of leaves produced per plant, root biomass,

root mass ratio (RMR), leaf mass ratio (LMR), Leaf:root ratio, root:leaf ratio, leaf width, leaf area ratio (LAR) and Specific leaf area (SLA). Biomass ratios and specific leaf area were calculated following Navas and Garnier (2002);

$$\text{RMR} = \text{Root biomass} / \text{Total biomass}$$

$$\text{LMR} = \text{Leaf biomass} / \text{Total biomass}$$

$$\text{LAR} = \text{Leaf area} / \text{Total biomass}$$

$$\text{SLA} = \text{Leaf area} / \text{Leaf biomass}$$

Flowers and seeds were also collected from the sampled plants, and oven dried to obtain their dry mass. This is, hereafter, referred to as Reproductive biomass.

### Data Analysis

Data were analyzed by the GLM approach to analysis of variance. We conducted separate analysis for each of the experiments using density or nutrient as fixed factors and the measured traits as the response variables. We tested for plasticity in trait expression at a given time i.e., final development stage (a stage common to all individual). We do not test for plasticity throughout growth and development (ontogenetic plasticity). To reveal adaptive plasticity we first considered and removed the possible confounding effect of plant size (Wright and McConnaughay, 2002). We started by testing for an

allometric relationship between plant total biomass and trait value for each treatment. Most of the traits showed a dependence on plant size, and so the effect of treatment was assessed by ANCOVA, using total biomass as a covariate (Ryser and Eek, 2000; Navas and Ganier, 2002). We used the raw data or log-transformed data when necessary in order to meet conditions of normality. When the effect of treatment was significant, the Fisher's LSD- procedure was used to compare means among treatments. We considered probability values of  $p < 0.05$  to be significant. The dependence of most of the traits on plant size made it necessary to calculate adjusted means before comparing the trait values (Ryser and Eek, 2000, Navas and Ganier, 2002). A significant total biomass term will indicate the presence of passive plasticity in a trait's expression (McConnaughay and Coleman, 1999). Similarly, a significant density or nutrient effect, after adjusting for the effect of total plant biomass (size) will indicate the presence of adaptive plasticity in a trait's expression (Weiner, 2004). Reproductive biomass was assessed by one-way analysis of variance (ANOVA). All analysis was done with SPSS Statistical Software (Version 11.5).

## RESULTS

Number of leaves, leaf width, leaf area ratio and specific leaf area were significantly affected by density (Table 1). Plants sown at high density produced more and wider leaves than the plants sown at medium and low densities (Table 2). Means comparisons showed that the values for these traits increased with increase in density (low < medium < High). However, the differences in leaf width were only significant between the plants sown at high and low density (Table 2). High density plants had significantly higher specific leaf area and leaf area ratio compared to the plants sown at medium and low densities (Table 2). Although density did not significantly affect plant height, plants sown at high density were taller compared with the other group of plants (Table 2). The differences in plant height was due to the effect of size (Significant total biomass term, Table 1). Means comparisons showed that plants sown at high density have higher leaf:root ratio compared to the other group of plants although the differences were due to plant size (Significant total biomass term (Table 1). Plants sown at high density however, have smaller leaf mass ratio compared with the other group of plants but the differences were not significant ( $p > 0.05$ , Table 2).

Table 1: Result of ANCOVA examining variation in the response traits to changes in resource availability.

Trait	Density		Nutrient	
	Covariate F	Treatment F	Covariate F	Treatment F
Plant height	5.671**	1.230NS	1.864NS	9.384**
No. Leaves	4.551**	16.377**	.187NS	8.087**
Leaf width	.998NS	4.649**	.000NS	6.750**
LAR	57.211**	7.786**	20.118**	4.852**
SLA	46.682**	6.518**	28.942**	1.535NS
RMR	12.367**	5.554**	1.701NS	3.563**
Root: Leaf	7.308**	.921NS	3.634NS	4.137**
RB	19.768**	1.356NS	23.950**	5.209**
LR	5.838**	.206NS	.006NS	.217NS

NS=not significant. See materials and methods for details of other abbreviations.

\*Significant \*\* Highly significant at  $p < 0.05$

Nutrient supply significantly affected most of the traits examined (Table 1). Low nutrient plants were significantly shorter with fewer leaves compared to the medium and high nutrient plants. In general, plant height and number of leaves increased with increase in nutrient supply (low nutrient < medium nutrient < High nutrient, Table 3). Leaf width and leaf length also differed significantly among treatments. Clearly, plants in the high nutrient treatment had significantly higher values compared to those sown at medium and low nutrients

(Table 3). This was followed by the plants sown at the medium nutrient treatment. However, the differences between the plants sown at medium and at low nutrient supply were not significant for these two traits (Table 3). Nutrient supply also significantly ( $p < 0.05$ ) affected root:leaf length, root biomass and root mass ratio (RMR). By contrast, plants sown at low nutrient supply had the highest values for these traits, with the lowest values obtained for the High nutrient plants (Low > Medium > High, Table 3).

Both treatments did not significantly affect Reproductive biomass (Fig 1).

TABLE 2: Effect of population density on growth and biomass allocation patterns in *Datura stramonium*

DENSITY VARIATION	Stem length(cm)	Leaf width(cm)	Specific leaf area	Number of leaves	Leaf area ratio	Leaf mass ratio	Leaf-root ratio
HIGH	23.01±1.05a	13.19±0.88a	2.67±0.07a	10.54±0.45a	2.25±0.55a	0.42±0.03a	5.80±0.97a
MEDIUM	20.74±1.05a	11.16±0.88a	2.40±0.07b	8.88±0.45b	2.08±0.55b	0.51±0.03a	5.09±0.97a
LOW	21.40±1.05a	9.40±0.88b	2.32±0.07b	6.91±0.45c	1.94±0.55b	0.43±0.03a	4.99±0.97a

Values are adjusted means correcting for the effect of plant size (see Materials and method, and Table 1). Means with the same letter are not significantly ( $p < 0.05$ ) different.

TABLE 3: Effect of nutrient on the growth and biomass allocation patterns in *Datura*

NUTRIENT AVAILABLE	Stem length (cm)	leave width (cm)	number of leaves	Leave area ratio	root: leaf ratio	root biomass	root mass ratio
HIGH	30.27±1.81a	13.24±0.87a	10.72±0.74a	2.44±0.11a	0.30±0.24b	0.12±0.03b	0.12±0.03b
MEDIUM	24.61±1.71b	11.04±0.82a	7.29±0.70b	2.13±0.10b	0.67±0.23a	0.17±0.03b	0.20±0.03a
LOW	18.97±1.74c	8.66±0.84b	6.71±0.71b	1.96±0.10b	1.29±0.23a	0.26±0.03a	0.25±0.03a

Values are adjusted means correcting for the effect of plant size (see Materials and method, and Table 1). Means with the same letter are not significantly ( $p < 0.05$ ) different.

## DISCUSSION

Plants sown at high densities, had significantly more leaves and higher leaf expansion, and were also marginally taller than those sown at medium and low density. High density is usually associated with

limited supply of light due to shading (Schmitt and Wulff, 1993; Ballaré and Scopel, 1997). In response to reduction in light quantity plants usually evolve some shade avoidance mechanism evident in elongation of the stems (Ballaré *et al.*, 1994; Leaflang *et al.*, 1998), increase in specific leaf area (Callaway *et al.*, 2003) and leaf area ratio (Navas and Garnier, 2002). This was precisely what was observed in this study. Increase in number of leaves, larger specific leaf area and leaf area ratio, as well as more expansion of leaves (leaf width) in response to high density would ameliorate the impact of low light because of increase in whole plant photosynthesis. Other potential benefits of increase in specific leaf area and leaf area ratio in response to high density include increase in plant competitive ability for light and reduction in degree of size asymmetry in competition (Schwinning and Weiner, 1998). At high densities plants are known to increase their heights in order to place their leaves in a better position for light interception, and therefore enhances the ability of such plants to better 'forage' for light (Weiner and Fishman, 1994).

Surprisingly, allocation to leaves (i.e. leaf mass ratio and leaf:root ratios) that are expected to increase with increase in density (Pooter and Nagel, 2000) only showed passive plasticity in response to density

(significant total biomass term). A number of studies have reported that stability in one trait can be achieved by plasticity in other traits (Bradshaw, 1965; Sultan and Bazzaz, 1993; Schlichting and Pigliucci, 1998). Thus the lack of adaptive plasticity in leaf mass ratio and leaf root ratio was compensated for by the plasticity in other traits such as specific leaf area and leaf area ratio. This therefore accentuates the importance of considering a wide range of traits to fully understand plant response to environmental conditions (Ryser and Eek, 2000). Studies examining plasticity in only few biomass traits in their interpretation of plasticity may likely underestimate the potential contribution of other changes that are also likely to be of functional value (Schlichting, 1989; Ryser and Eek, 2000).

Nutrient availability also led to significant plastic responses in this study. When size-adjusted means were compared, the low nutrients supplied plants had significantly higher root biomass and root mass ratio (RMR), and significantly higher root:leaf ratio compared to the medium and high nutrient plants. Even when we accounted for possible size effect, these differences were still significant. By contrast the low nutrient plants were significantly shorter with narrower leaves as indicated by their leaf width and smaller leaf area ratios. This



indicates adaptive plastic investments in nutrients acquiring traits instead of traits responsible for light capture (Bloom *et al.*, 1985). Appreciable numbers of studies have also reported increased allocation to roots in response to low nutrient availability (Gersani *et al.*, 2001). The significant reduction in light acquiring traits such as plant height, leaf width and leaf area ratio in the nutrient treatment suggest that these traits are not of clear adaptive value under the shortage of a soil resource like nutrient (Navas and Garnier, 2002).

Reproductive biomass was not significantly affected by both density and nutrient supply (Fig 1). For an annual seed producing plant, fitness gained during growth and development should maximize the number of seeds produced at the end of the growing season (Bonser and Aarssen, 2003). In our study, plants exposed to unfavorable environment (High density and Low nutrient) were not adversely affected in terms of seed production probably due to the plasticity exhibited during growth.

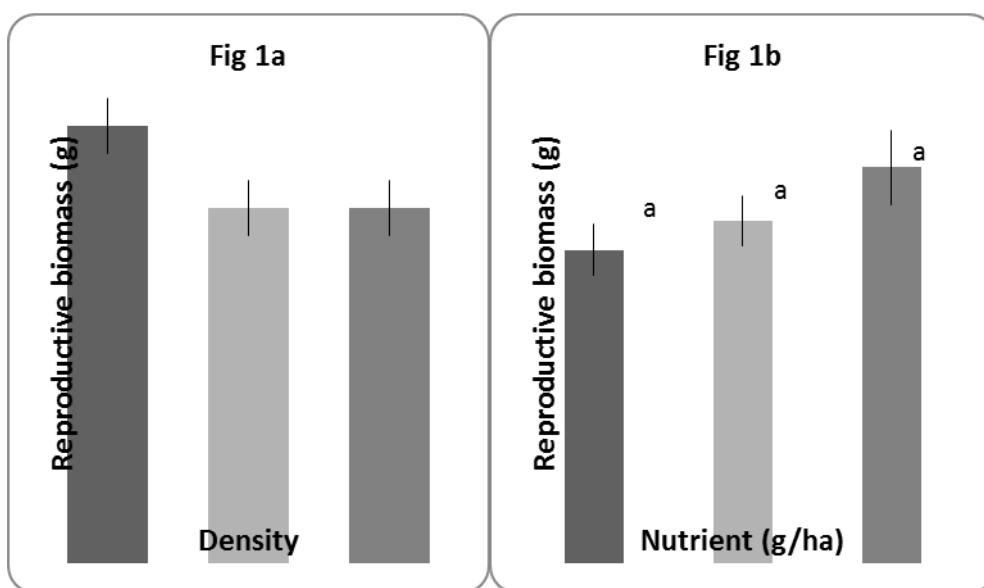


Fig 1: Effect of (a) density and (b) nutrient supply on Reproductive biomass in *Datura stramonium*. Bars are standard error bars.

In conclusion, our study demonstrates significant plasticity in appreciable number of traits that might enhance the survival and growth of *Datura stramonium* in heterogeneous environments. The ability of a species to maximize fitness in response to limited resource has important implication in determining the range of habitat such species can colonize (Bonser and Aarssen, 2003), and such information is crucial in developing any management strategy and predictive model of its spread.

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